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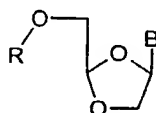
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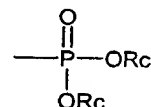
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(54) Title: METHODS OF TREATING LEUKEMIA



(I)



(a)

(57) Abstract

The present invention provides a novel method for treating leukemia and more particularly acute myelogenous leukemia (AML) in a host comprising administering to the host a therapeutically effective amount of a compound having formula (I): wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and (a) wherein each Rc is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group; and wherein said compound is substantially in the form of the (-) enantiomer.

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METHODS OF TREATING LEUKEMIA

Field of the invention

The present invention relates to methods for treating leukemia, and more particularly, to the use of nucleoside analogues as an effective treatment for acute or chronic myelogenous leukemia.

Background of the Invention

Leukemia is a malignant cancer of the bone marrow and blood. It is characterized by the uncontrolled growth of blood cells. The common types of leukemia are divided into four categories: acute or chronic myelogenous, involving the myeloid elements of the bone marrow (white cells, red cells, megakaryocytes) and acute or chronic lymphocytic, involving the cells of the lymphoid lineage.

Acute leukemia is a rapidly progressing disease that results in the massive accumulation of immature, functionless cells (blasts) in the marrow and blood. The marrow often can no longer produce enough normal red and white blood cells and platelets. Anemia, a deficiency of red cells, develops in virtually all leukemia patients. The lack of normal white cells impairs the body's ability to fight infections. A shortage of platelets results in bruising and easy bleeding. In contrast, chronic leukemia progresses more slowly and leads to unregulated proliferation and hence marked overexpansion of a spectrum of mature (differentiated) cells. In general, acute leukemia, unlike the chronic form, is potentially curable by elimination of the neoplastic clone.

It is estimated that there will be 28,700 new cases of leukemia in the United States this year; about equal

proportions are acute leukemia and chronic types. Most cases occur in older adults. Leukemia is expected to strike ten times as many adults as children in 1998. (About 26,500 cases compared to 2,200 in children) More than half of all cases of leukemia occur in persons over 60. The most common types of leukemia in adults are acute myelogenous leukemia (AML) with an estimated 9,400 new cases annually, chronic lymphocytic leukemia (CLL), with some 7,300 new cases this year and chronic myeloid leukemia (CML). The most common type of leukemia in children is acute lymphocytic leukemia (ALL).

Standard treatment for leukemia usually involves chemotherapy and /or bone marrow transplantation and/or radiation therapy.

The two major types of bone marrow transplants are autologous (uses the patient's own marrow) and allogeneic (uses marrow from a compatible donor). Radiation therapy, which involves the use of high-energy rays, is usually given before bone marrow transplantation to kill all leukemic cells.

Chemotherapy in leukemia usually involves a combination of two or more anti-cancer drugs. Approximately 40 different drugs are now being used in the treatment of leukemia. Some common combinations include cytarabine with either doxorubicin or daunorubicin or mitoxantrone or thioguanine, mercaptopurine with methotrexate, mitoxantrone with etoposide, asparaginase with vincristine, daunorubicin and prednisone, cyclophosphamide with vincristine, cytarabine and prednisone, cyclophosphamide with vincristine and prednisone, daunorubicin with cytarabine and thioguanine and daunorubicin with vincristine and prednisone.

New treatments for leukemia also include the reversal of multidrug resistance, involving the use of agents which decrease the mechanisms allowing the malignant cells to escape

the damaging effects of the chemotherapeutic agent (and leads to refractoriness or relapses); and biological therapy, involving the use of substances known as biological response modifiers (BRMs). These substances are normally produced in small amounts as part of the body's natural response to cancer or other diseases. Types of BRMs include monoclonal antibodies, in which toxins are attached to antibodies that react with the complementary antigen carried by the malignant cells; and cytokines (e.g. interferons, interleukins, colony-stimulating factors CSFs) which are naturally occurring chemicals that stimulate blood cell production and help restore blood cell counts more rapidly after treatment. Examples of these drugs include multidrug resistance reversing agent PSC 833, the monoclonal antibody Rituxan and the following cytokines: Erythropoietin and Epoetin, which stimulate the production of red cells; G-CSF, GM-CSF, filgrastim, and Sargramostim which stimulate the production of white cells; and thrombopoietin, which stimulate the production of platelets.

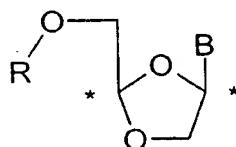
Many nucleoside analogues have been found to possess anticancer cancer activity. Cytarabine, Fludarabine, Gemcitabine and Cladribine are some examples of nucleoside analogues which are currently important drugs in the treatment of leukemia.

(-)- β -L-Dioxolane-Cytidine (β -L-OddC) is also a nucleoside analogue that was first described as an antiviral agent by Belleau et al. (EP 337713) and has been shown to have potent antitumor activity (K.L. Grove et al., Cancer Res., 55(14), 3008-11, 1995; K.L. Grove et al., Cancer Res., 56(18), 4187-4191, 1996; K.L. Grove et al., Nucleosides Nucleotides, 16:1229-33, 1997; S.A Kadhim et al., Can. Cancer Res., 57(21), 4803-10, 1997).

Treatment of leukemia is very complex and depends upon the type of leukemia. Tremendous clinical variability among remissions is also observed in leukemic patients, even those that occur after one course of therapy. Patients who are resistant to therapy have very short survival times, regardless of when the resistance occurs. Despite improvements in outcome with current treatment programs, the need to discover novel agents for the treatment of all types of leukemia continues.

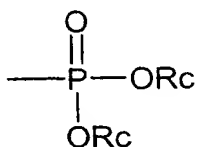
Summary of the Invention

The present invention provides a novel method for treating leukemia in a host comprising administering a therapeutically effective amount of a compound having the formula I:



(I)

wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising (H), monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each Rc is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group; and

wherein said compound is substantially in the form of the (-) enantiomer.

In another embodiment, there is provided a method for treating leukemia in a host comprising administering to the host a therapeutically effective amount of at least one compound according to formula I and at least one further therapeutic agent selected from the group comprising chemotherapeutic agents; multidrug resistance reversing agents; and biological response modifiers.

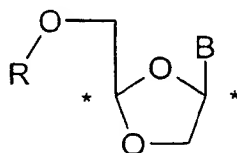
Still another embodiment, there is provided a pharmaceutical composition for treating leukemia comprising at least one compound according to formula I together with at least one pharmaceutically acceptable carrier or excipient.

In another embodiment, there is provided a pharmaceutical composition for treating leukemia comprising at least one compound according to formula I and at least one further therapeutic agent selected from the group comprising chemotherapeutic agents; multidrug resistance reversing agents; and biological response modifiers.

In another embodiment of the invention is the use of a compound according to formula I for the manufacture of a medicament for treating leukemia.

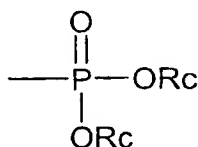
Detailed Description of the Invention

The present invention provides a novel method for treating leukemia in a host comprising administering a therapeutically effective amount of a compound having the formula I:



(I)

wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each Rc is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group; and

wherein said compound is substantially in the form of the (-) enantiomer.

In another embodiment of the invention, R is H.

In another embodiment, B is cytosine.

Alternatively, in another embodiment, B is 5-fluorocytosine.

In one embodiment, a compound of formula I is (-)-β-L-Dioxolane-Cytidine (β-L-oddC).

In another embodiment, a compound of formula I is (-)-β-Dioxolane-5-fluoro-Cytidine (5-FddC)

It will be appreciated by those skilled in the art that the compounds of formula (I) contain at least two chiral centres which are marked by an asterisk (*) on formula (I). The compounds of formula (I) thus exist in the form of two

different optical isomers (i.e. (+) or (-) enantiomers or β -L and β -D). All such enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. The single optical isomer or enantiomer can be obtained by method well known in the art, such as chiral HPLC, enzymatic resolution and the use of chiral auxiliary.

According to one embodiment, compounds of formula I of the present invention are provided substantially in the form of the (-) enantiomer.

By "substantially" is meant that there is more of the (-) enantiomer than the (+) enantiomer.

In another embodiment, the compounds of formula I of the present invention are at least 95% free of the corresponding (+) enantiomer.

In another embodiment, the compounds of formula I of the present invention are at least 97% free of the corresponding (+) enantiomer.

Still in another embodiment, the compounds of formula I of the present invention are at least 99% free of the corresponding (+) enantiomer.

There is also provided pharmaceutically acceptable salts of the compounds of formula I of the present invention. By the term pharmaceutically acceptable salts of the compounds of formula (I) are meant those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric,

acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4} alkyl) salts.

As used in this application, the term "alkyl" represents an unsubstituted or substituted (by a halogen, nitro, CONH_2 , COOH , O-C_{1-6} alkyl, O-C_{2-6} alkenyl, O-C_{2-6} alkynyl, hydroxyl, amino, or COOQ , wherein Q is C_{1-6} alkyl; C_{2-6} alkenyl; C_{2-6} alkynyl) straight chain, branched chain or cyclic hydrocarbon moiety (e.g. isopropyl, ethyl, fluorohexyl or cyclopropyl). The term alkyl is also meant to include alkyls in which one or more hydrogen atoms is replaced by an halogen, more preferably, the halogen is fluoro (e.g. CF_3 - or CF_3CH_2 -).

The terms "alkenyl" and "alkynyl" represent an alkyl containing at least one unsaturated group (e.g. allyl).

The term "hydroxy protecting group" is well known in the field of organic chemistry. Such protecting groups may be found in T. Greene, Protective Groups In Organic Synthesis, (John Wiley & Sons, 1981). Example of hydroxy protecting groups include but are not limited to acetyl-2-thioethyl ester, pivaloyloxymethyl ester and isopropylloxycarbonyloxymethyl ester.

The term "aryl" represent an unsaturated carbocyclic moiety, optionally mono- or di-substituted with OH, SH, amino, halogen or C_{1-6} alkyl, and optionally substituted by at least one heteroatom (e.g. N, O, or S).

In one embodiment, the present invention provides a method for treating myelogenous leukemia.

In another embodiment, the present invention provides a novel method for treating acute myelogenous leukemia.

In another embodiment, the present invention provides a novel method for treating chronic myelogenous leukemia.

Still in another embodiment, the present invention provides a novel method for treating multidrug resistant leukemia.

The term "leukemia" represent acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL) and all subtypes of these leukemias which are defined by morphological, histochemical and immunological techniques that are well known by those skilled in the art.

The term "myelogenous leukemia" represent both acute and chronic myelogenous leukemias (AML, CML) which involve the myeloid elements of the bone marrow (e.g. white cells, red cells and megakaryocytes) and includes all subtypes which are defined by morphological, histochemical and immunological techniques that are well known by those skilled in the art.

The term "multidrug resistant leukemia" represent a leukemia which is non responsive to treatment with chemotherapeutic agents.

The term "host" represent any mammals including humans.

In one embodiment, the host is human.

According to one embodiment, the patient treated has been previously treated with cytarabine (Ara-C). The patient is treated according to any one of the method set forth herein.

According to one embodiment, the patient that has been previously treated is resistant to cytarabine (Ara-C). The patient is treated according to any one of the methods set forth herein.

According to another embodiment, the patient is refractory to Ara-C.

According to one embodiment, it will be appreciated that the amount of a compound of formula I of the present invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition for which treatment is required and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.01 to about 750 mg/kg of body weight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20 mg/kg/day.

The desired dose according to one embodiment is conveniently presented in a single dose or as divided dose administered at appropriate intervals, for example as two, three, four or more doses per day.

In another embodiment, the compound is conveniently administered in unit dosage form; for example containing 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient per unit dosage form.

According to another embodiment of the present invention, the active ingredient is administered to achieve peak plasma concentrations of the active compound of from about 1 to about 75 μ M, preferably about 2 to 50 μ M, most preferably about 3 to

about 30 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1 to about 500 mg of the active ingredient. Desirable blood levels may be maintained by a continuous infusion to provide about 0.01 to about 5.0 mg/kg/hour or by intermittent infusions containing about 0.4 to about 15 mg/kg of the active ingredient.

While it is possible that, for use in therapy, a compound of formula I of the present invention may be administered as the raw chemical, it is preferable according to one embodiment of the invention, to present the active ingredient as a pharmaceutical formulation. The embodiment of the invention thus further provides a pharmaceutical formulation comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

According to one embodiment of the present invention, pharmaceutical formulations include but are not limited to those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), transdermal, vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods according to this embodiment include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers

or both and then, if necessary, shaping the product into the desired formulation.

According to another embodiment, pharmaceutical formulation suitable for oral administration are conveniently presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules. In another embodiment, the formulation is presented as a solution, a suspension or as an emulsion. Still in another embodiment, the active ingredient is presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compounds of formula I according to an embodiment of the present invention are formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis, the compounds of formula I, according to one embodiment of the present invention, are formulated as ointments, creams or lotions, or as a transdermal patch. Such transdermal patches may contain penetration enhancers such as linalool, carvacrol, thymol, citral, menthol and t-anethole. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid. In another embodiment, they are presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

According to one embodiment, the formulations suitable for vaginal administration are presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For intra-nasal administration the compounds, in one embodiment of the invention, are used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one more dispersing agents, solubilising agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.

For administration by inhalation the compounds, according to one embodiment of the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. In another embodiment, pressurized packs comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In another embodiment, the dosage unit in the pressurized aerosol is determined by providing a valve to deliver a metered amount.

Alternatively, in another embodiment, for administration by inhalation or insufflation, the compounds of formula I according to the present invention are in the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. In another embodiment, the powder composition is presented in unit dosage form in, for example, capsules or cartridges or e.g. gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

In one embodiment, the above described formulations are adapted to give sustained release of the active ingredient.

In another embodiment, there is provided a method for treating leukemia in a host comprising administering to the host a therapeutically effective amount of at least one compound

according to formula I and at least one further therapeutic agent selected from the group comprising chemotherapeutic agents; multidrug resistance reversing agents; and biological response modifiers.

In another embodiment, the chemotherapeutic agents are selected from the group consisting of Asparaginase, Bleomycin, Busulfan, Carmustine, Chlorambucil, Cladribine, Cyclophosphamide, Cytarabine, Dacarbazine, Daunorubicin, Doxorubicin, Etoposide, Fludarabine, Gemcitabine, Hydroxyurea, Idarubicin, Ifosfamide, Lomustine, Mechlorethamine, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitoxantrone, Pentostatin, Procarbazine, 6-Thioguanine, Topotecan, Vinblastine, Vincristine, Dexamethasone, Retinoic acid and Prednisone.

In another embodiment, the chemotherapeutic agents are selected from the group consisting of Cytarabine, Etoposide, Mitoxantron, Cyclophosphamide, Retinoic acid, Daunorubicin, Doxorubicin and Idarubicin.

Still in another embodiment, the chemotherapeutic agent is Doxorubicin.

In one embodiment, the multidrug resistance reversing agent is PSC 833.

In another embodiment, the biological response modifiers are selected from the group consisting of monoclonal antibodies and cytokines.

In another embodiment, the cytokines are selected from the group consisting of interferons, interleukins and colony-stimulating factors.

In another embodiment, the biological response modifiers are selected from the group consisting of Rituxan, CMA-676, Interferon-alpha recombinant, Interleukin-2, Interleukin-3, Erythropoetin, Epoetin, G-CSF, GM-CSF, Filgrastim, Sargramostim and Thrombopoietin.

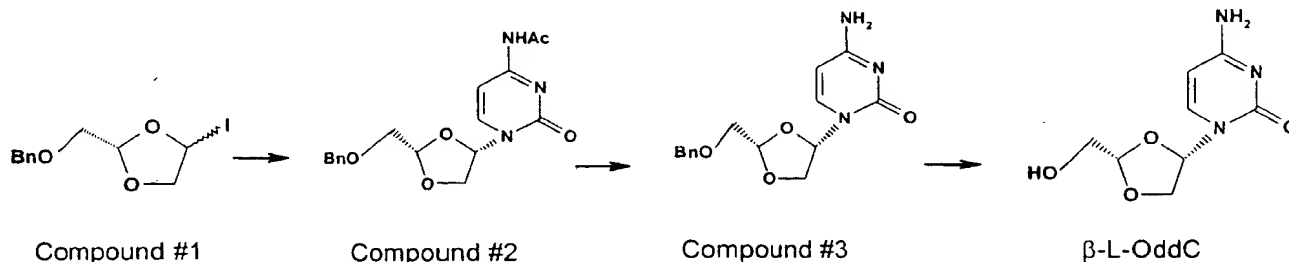
In one embodiment of the present invention, the combinations referred to above are conveniently presented for use in the form of a pharmaceutical composition comprising a combination as defined above together with a pharmaceutically acceptable carrier.

In another embodiment, the individual components of such combinations are administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

In one embodiment of the present invention, when the compound of formula I or a pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent, the dose of each compound is either the same as or differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compounds of formula I of the present invention can be prepared as follows.

The following examples are provided to illustrate various embodiments of the present invention and shall not be considered as limiting in scope.

Example 1. Preparation of β -L-oddC.Scheme 1

Compound #1: 2S-Benzyloxymethyl-4R-iodo-1,3 dioxolane and
2SBenzyloxymethyl-4S-iodo-1,3 dioxolane

A mixture consisting of 2S-benzyloxymethyl-4S acetoxymethyl-1,3 dioxolane and 2S-benzyloxymethyl-4R-acetoxymethyl-1,3 dioxolane in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic distillation with toluene in vacuo. After removal of toluene, the residual oil was dissolved in dry dichloromethane (60 ml) and iodotrimethylsilane (3.55 ml; 1.05 eq) was added at -78°C , under vigorous stirring. The dry-ice/acetone bath was removed after addition and the mixture was allowed to warm up to room temperature (15 min.). The ^1H NMR indicated the formation of 2S-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2S-benzyloxymethyl-4S-iodo-1,3 dioxolane.

^1H NMR (300 MHz, CDCl_3) δ 3.65-4.25 (2H,m); 4.50-4.75 (4H,m)
 5.40-5.55 (1H, overlapping triplets); 6.60-6.85 (1H, d of d);
 7.20-7.32 (5H,m).

Compound #2: β -L-5'-Benzyl-2'-deoxy-3'-oxa-N-4-acetyl-cytidine

The previously prepared iodo intermediate (Compound #1) in dichloromethane, was cooled down to -78°C . Persilylated N-acetyl cytosine (1.1 eq) formed by reflux in 1,1,1,3,3,3-hexamethyl disilazane (HMDS) and ammonium sulphate followed by evaporation of HMDS was dissolved in 30 ml of dichloromethane and was added to the iodo intermediate. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2 x 25 ml).

The organic phase was dried over sodium sulphate, the solid was removed by filtration and the solvent was evaporated in vacuo to produce 8.1 g of a crude mixture. Based on ^1H NMR analysis, the β -L-5'-benzyl-2'-deoxy-3'-oxacytidine and its α -L isomer were formed in a ratio of 5:1 respectively. This crude mixture was separated by chromatography on silica-gel (5% MeOH in EtOAc) to generate the pure β -L (cis) isomer (4.48 g). Alternatively, recrystallization of the mixture from ethanol produces 4.92 g of pure β isomer and 3.18 g of a mixture of β and α -isomers in a ratio of 1:1. ^1H NMR (300 MHz, CDCl_3) δ 2.20 (3H, s, Ac); 3.87 (2H, m, H-5'), 4.25 (2H, m, H-2'); 4.65 (2H, dd, OCH_2Ph); 5.18 (1H, t, H-4'); 6.23 (1H, m, H-1'); 7.12 (1H, d, H-5); 7.30-7.50 (5H, m, Ph); 8.45 (2H, m, NH+H-6).

Compound #3: β -L-5'-Benzyloxy-2'-deoxy-3'-oxacytidine

The protected β -L isomer (4.4 g) (Compound #2) was suspended in saturated methanolic ammonia (250 ml) and stirred at room temperature for 18 hours in a closed vessel. The solvents were then removed in vacuo to afford the deacetylated nucleoside in pure form.

^1H NMR (300 MHz, CDCl_3) δ 3.85 (2H, m, H-5'); 4.20 (2H, m, H-2'); 4.65 (2H, dd, OCH_2Ph); 5.18 (1H, t, H-4'); 5.43 (1H, d, H-5); 5.50-

5.90 (2H, br. S, NH₂); 6.28 (1H, m, H-1'); 7.35-7.45 (5H, m, Ph);
7.95 (1H, d, H-6).

Compound #4: β -L-OddC

β -L-5'-Benzyl-2'-deoxy-3'-oxacytidine (Compound #3) was dissolved in EtOH (200 ml) followed by addition of cyclohexene (6 ml) and palladium oxide (0.8 g). The reaction mixture was refluxed for 7 hours then it was cooled and filtered to remove solids. The solvents were removed from the filtrate by vacuum distillation. The crude product was purified by flash chromatography on silica-gel (5% MeOH in EtOAc) to yield a white solid (β -L-OddC) (2.33 g; 86% overall yield, $\alpha_D^{22} = -46.7^\circ$ (c = 0.285; MeOH) m.p. = 192 - 194°C). ¹H NMR (300 MHz, DMSO-d₆) δ 3.63 (2H, dd, H-5'); 4.06 (2H, m, H-2'); 4.92 (1H, t, H-4'); 5.14 (1H, t, OH); 5.70 (1H, d, H-5); 6.16 (2H, dd, H-1'); 7.11 - 7.20 (2H, br S, NH₂); 7.80 (1H, d, H-6) ¹³C NMR (75 MHz, DMSO-d₆) δ 59.5 (C-2'); 70.72 (C-5'); 81.34 (C-4'); 93.49 (C-1'); 104.49 (C-5); 140.35 (C-4); 156.12 (C-6); 165.43 (C-2).

Example 2. Evaluation of β -L-oddC in patients with advanced leukemia.

The study involved treatment of patients with advanced leukemia that had been previously treated with Citarabine (Ara-C). The previous treatment with Ara-C had failed to stop progression of the disease. Twelve patients were treated with an initial course at daily doses of 0.72 mg/m² (4 patients), 1.08 mg/m² (5 patients), 1.62 mg/m² (3 patients) given as a daily infusion over 30 minutes for 5 consecutive days. Five patients were treated with second courses at daily doses of 1.08 mg/m² (3 patients), 1.62 mg/m² (2 patients) given over 5 consecutive days as above. 1 patient was treated with a 3rd 5 day course at the 2.43 mg/m² daily dose level. 4 patients (2 each at the 1.08 mg/m² and 1.62 mg/m² levels) have shown a transient decrease in peripheral blood and bone marrow blasts. Of these four patients, three had acute myelogenous leukemia and one had chronic myelogenous leukemia.

Example 3. β -L-OddC /doxorubicine combination study in a human leukemia (HL60) xenograft model

A study was conducted to evaluate the synergistic or additive therapeutic effect of β -L-OddC in combination with the currently known anticancer agent Doxorubicin. The model that was utilized is a survival model consisting of female SCID mice which are inoculated in the abdomen region (i.p.) with 15×10^6 HL60 cells in log phase growth. This corresponds to day 0 of the experiment. Administration of anti-cancer drug is started 10 days after tumor cell inoculation.

10 animals were used per group for β -L-oddC alone, Doxorubicin alone and the combination of β -L-oddC with Doxorubicin. Each

groups received the drugs alone or in combination intravenously once daily for 5 consecutive days.

Augmentation of survival time was calculated by subtracting from the median survival time of group two to six, which corresponds to the day when the fifth mouse dies, the median survival time of control group 1 and multiplying by 100.

In Table 1 below, we observe that the best treatment corresponds to the combination of β -L-oddC with Doxorubicin at a dose of 2 mg/Kg. This combination extends the survival time of the mice substantially compared to either single agents β -L-oddC and Doxorubicin.

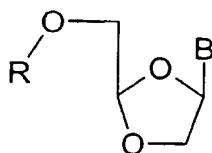
TABLE 1. COMBINATION STUDY β -L-oddC /DOXORUBICIN IN HUMAN LEUKEMIA (HL60)

Group of	Combination	Augmentation Survival Time
1	Saline i.p.	
2	β -L-OddC 1mg/kg	55%
3	Doxorubicin 0.2mg/kg	25%
4	β -L-OddC 1mg/kg + Doxorubicin 0.2mg/kg	55%
5	Doxorubicin 2mg/kg	50%
6	β -L-OddC 1mg/kg. + Doxorubicin 2mg/kg	100%

We claim

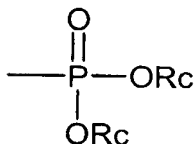
1. A method for treating a patient with leukemia in a host comprising:

administering to a patient having chronic myelogenous leukemia or acute myelogenous leukemia, a therapeutically effective amount of a compound having the formula I:



(I)

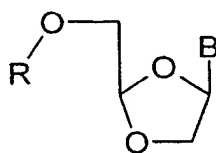
wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each Rc is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group; and wherein said compound is substantially in the form of the (-) enantiomer.

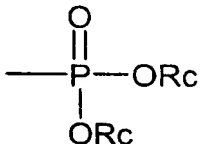
2. The method according to claim 1, wherein the step of administering comprises administering to a patient that has been previously treated with Ara-C.
3. The method according to claim 2, wherein R is H.
4. The method according to claim 2, wherein B is cytosine.

5. The method according to claim 2, wherein R is H and B is cytosine.
6. The method according to claim 4, wherein said compound of formula I is at least 95% free of the (+) form.
7. The method according to claim 4, wherein said compound of formula I is at least 97% free of the (+) form.
8. The method according to claim 4, wherein said compound of formula I is at least 99% free of the (+) form.
9. The method of claim 2, wherein the leukemia is a chronic myelogenous leukemia.
10. The method of claim 2, wherein the leukemia is an acute myelogenous leukemia.
11. A method for treating leukemia in a host comprising administering to the host having leukemia a therapeutically effective amount of at least one compound of general formula I



(I)

wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each R_c is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group, and wherein said compound is substantially in the form of the (-) enantiomer; and

administering doxorubicin to a patient.

12. The method according to claim 11, wherein the leukemia is chronic myelogenous leukemia.

13. The method according to claim 11, wherein the leukemia is acute myelogenous leukemia.

14. The method according to claim 11, further comprising the step of administering a multidrug resistance reversing agent or a biological response modifier.

15. The method according to claim 14, wherein the multidrug resistance agent is PSC 833.

16. The method according to claim 14, wherein the biological response modifiers are selected from the group consisting of monoclonal antibodies and cytokines.

17. The method according to claim 14, wherein the cytokines are selected from the group consisting of interferons, interleukins and colony-stimulating factors.

18. The method according to claim 14, wherein the biological response modifiers are selected from the group consisting of Rituxan, CMA-676, Interferon-alpha recombinant, Interleukin-2, Interleukin-3, Erythropoietin, Epoetin, G-CSF, GM-CSF, Filgrastim, Sargramostim and Thrombopoietin.

19. The method according to claim 11, wherein the compound of formula I and the doxorubicin are administered sequentially.
20. The method according to claim 11, wherein the compound of formula I and the doxorubicin are administered simultaneously.

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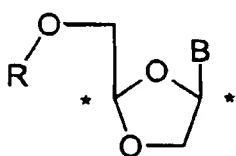
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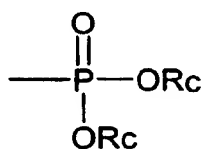
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For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: USE OF CYTIDINE DERIVATIVES FOR THE TREATMENT OF LEUKAEMIA



(I)



(a)

(57) Abstract: The present invention
provides a novel method for treating
leukemia and more particularly acute
myelogenous leukemia (AML) in
a host comprising administering to
the host a therapeutically effective
amount of a compound having formula

(I): wherein B is cytosine or 5-fluorocytosine and R is selected from the group comprising H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and (a) wherein each Rc is independently selected from the group comprising H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group; and wherein said compound is substantially in the form of the (-) enantiomer.

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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 96 07413 A (UNIV GEORGIA) 14 March 1996 (1996-03-14) page 6, line 12-25 page 6, line 29 -page 8, line 11 page 12-15; claims 1-10,12,23-25,27,38-42; examples 9,12; table 2 ---	1-8,11, 14,16-20 9,10,12, 13
A	EP 0 337 713 A (IAF BIOCHEM INT) 18 October 1989 (1989-10-18) page 2, line 9-11 page 3, line 40 -page 4, line 1 page 4, line 23-26 page 4, line 34 -page 5, line 22 page 5, column 31-41; claims 1,4,5,11,13-15 --- -/-	1,3-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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G document member of the same patent family

Date of the actual completion of the international search

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Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 382 526 A (IAF BIOCHEM INT) 16 August 1990 (1990-08-16) page 3, line 35-65; claims 1,2,4,5,11 page 4, line 23-25; claim 13 page 4, line 35-54 page 6 -page 7, line 18 page 9, line 6-12 page 11, column 2-6 -----	1,3-14

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Present claims 14 and 16-18 relate to methods involving compounds defined by reference to desirable characteristics or properties, namely reversing multidrug resistance and colony-stimulating.

The claims cover all compounds having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). A compound cannot be adequately defined by its mechanism of action and/or its pharmacological profile. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Furthermore, present claims 14-17 relate to methods involving an extremely large number of possible compounds. Due thereto, a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the complete scope of the claims impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear (and/or concise), supported and disclosed, namely those parts relating to the compounds defined in present claims 15 and 18 and in the examples; with due regard to the description and the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 00/00334

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9607413 A	14-03-1996	US 5817667 A	06-10-1998
		AP 783 A	17-11-1999
		AU 704977 B	13-05-1999
		AU 3586295 A	27-03-1996
		BG 101284 A	31-03-1998
		BR 9508886 A	30-12-1997
		CA 2199117 A	14-03-1996
		CN 1160351 A	24-09-1997
		CZ 9700633 A	16-07-1997
		EP 0781136 A	02-07-1997
		FI 970918 A	02-05-1997
		HU 77172 A	02-03-1998
		JP 10506385 T	23-06-1998
		PL 318971 A	21-07-1997
		SK 28197 A	10-09-1997
		US 6063787 A	16-05-2000
		NO 971015 A	05-03-1997
EP 0337713 A	18-10-1989	AT 129247 T	15-11-1995
		AU 631786 B	10-12-1992
		AU 3264489 A	12-10-1989
		CA 1339609 A	30-12-1997
		DE 68924549 D	23-11-1995
		DE 68924549 T	04-04-1996
		DK 172089 A	12-10-1989
		ES 2078234 T	16-12-1995
		GR 3017812 T	31-01-1996
		HK 5996 A	19-01-1996
		IE 71225 B	12-02-1997
		IL 89921 A	18-08-1993
		JP 1316375 A	21-12-1989
		JP 3085675 B	11-09-2000
		KR 137023 B	25-04-1998
		NZ 228645 A	25-09-1991
		OA 9470 A	15-11-1992
		US 5684164 A	04-11-1997
		US 5041449 A	20-08-1991
		US 5270315 A	14-12-1993
		ZA 8902645 A	27-12-1989
EP 0382526 A	16-08-1990	CA 2152269 A	07-07-1994
		US 5047407 A	10-09-1991
		AP 136 A	05-08-1991
		AT 138065 T	15-06-1996
		AU 3154993 A	19-07-1994
		AU 630913 B	12-11-1992
		AU 4920190 A	16-08-1990
		CA 2009637 A, C	08-08-1990
		CN 1044817 A, B	22-08-1990
		CS 9104109 A	15-07-1992
		CY 2036 A	20-02-1998
		DE 69026971 D	20-06-1996
		DE 69026971 T	12-09-1996
		DK 382526 T	05-08-1996
		EP 0674634 A	04-10-1995
		EP 0711771 A	15-05-1996
		ES 2086371 T	01-07-1996
		FI 98065 B	31-12-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 00/00334

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0382526 A		GR 3019919 T	31-08-1996
		HK 51997 A	02-05-1997
		HR 940040 A	30-04-1997
		HU 210537 B	28-04-1995
		IE 72184 B	26-03-1997
		IL 93318 A	31-07-1995
		JP 2644357 B	25-08-1997
		JP 3007282 A	14-01-1991
		JP 8119967 A	14-05-1996
		JP 8504212 T	07-05-1996
		KR 9607531 B	05-06-1996
		LU 88809 A	03-01-1997
		MX 19437 A	01-05-1993
		NO 179518 B	15-07-1996
		OA 9193 A	30-06-1992
		PL 164785 B	31-10-1994
		PT 93094 A, B	31-08-1990
		SI 9010243 A, B	31-10-1996
		RU 2092485 C	10-10-1997
		US 5466806 A	14-11-1995
		HU 53362 A, B	28-10-1990
		NZ 232421 A	26-10-1993
		SG 48737 A	18-05-1998
		SK 279175 B	08-07-1998
		US 5684164 A	04-11-1997
		US 5151426 A	29-09-1992
		YU 24390 A	31-10-1991
		ZA 9000943 A	31-10-1990